REMARKS

Overview

Claims 1-7 and 14-29 are pending in this application. Claims 8-13 have been cancelled. Claims 1, 5 and 6 have been amended. The present response is an earnest effort to place the present application in proper form for allowance.

Specification - Objections

Figure legend 4 is objected to. The Examiner states:

> The legend describes 4A and 4B as being the same, but the data in the Specification indicate that 4A and 4B are different.

PTO Paper dated September 23, 2003 at p. 2.

In the specification, on page 5, fourth paragraph under "Brief Description of the Drawings", the paragraph has been amended to be in accordance with the data in the specification taken from the paragraph bridging pages 8-9 of the specification. Therefore, it is respectfully submitted that this rejection should be withdrawn.

Claims - Objections

Claim 6 has been objected to for having "of" on line 7 in the phrase "expressing of the recombinant". Therefore, Applicants have amended claim 6 by deleting "of" in line 7, thus making this objection moot.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 1-7 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner states:

> Regarding the phrase "carboxy terminal portion of a fluorescent reporter" on line 4, Claims 1 and 6 have two interpretations. Said carboxy terminal portion could be derived from the same fluorescent reporter having the "amino terminal portion" as recited in line 2 of claims 1 and 6. Alternatively, the N- and C-terminal portions could be derived from either the same or different fluorescent reporters. Claims 2-5 and 7, as dependent from claims 1 and 6, respectively, are rejected for the same reasons. For purposes of examination, it is assumed that the N- and C-terminal portions are derived from the same fluorescent reporter. It is suggested that Applicants amend the "a fluorescent" on line 4 to "the fluorescent.

Id. at page 3.

Applicants have amended claims 1 and 6 to recite "carboxyl terminal portion of the fluorescent reporter" to more clearly indicate that the N- and C-terminal portions are derived from the same fluorescent reporter. Applicants thank the Examiner for this suggestion.

Claims 2-5 and 7 as dependent on claims 1 and 6, respectively contain by virtue of their dependency all the limitations of amended independent claims 1 and 6. Reconsideration is respectfully requested.

Next, the Examiner states:

> Regarding the phrase "expressing a recombinant fluorescent substrate" on line 6, claim 1 has two interpretations. Said recombinant fluorescent substrate could be the fusion protein described in lines 1-5 of Claim 1. Alternatively, said recombinant fluorescent substrate could encompass any fluorescent substrate, including the fusion protein described in lines 1-5 of Claim 1. Claims 2-5, as dependent from Claim 1, are rejected for the same reasons. For purposes of examination, it is assumed that the recombinant fluorescent substrate is the fusion protein described in lines 1-5 of Claim 1. It is suggested that applicants amend the "a recombinant" on line 6 to "the recombinant.

Id.

Applicants have amended claim 1 to recite "the recombinant" in line 6 to more clearly indicate that the recombinant fluorescent substrate is the fusion protein described in lines 1-5 of

the claim. Dependent claims 2-5 by virtue of their dependency contain all the limitations of amended claim 1. Applicants respectfully request that this rejection be withdrawn.

The Examiner states:

> In Claims 1 and 6, "A method of assaying for protease activity, comprising providing a nucleic acid construct" is unclear. Nucleic acid molecules are not protease substrates. The described construct would have to be translated and the encoded protein used as a substrate. Claims 2-5 and 7, as dependent from claims 1 and 6, respectively are rejected for the same reason. For purposes of examination, it is assumed that the nucleic acid construct is transfected into host cells for production of the encoded substrate.

Id. at pp. 3-4.

Applicants have amended claims 1 and 6 to indicate that the screening for protease activity is inside cells. This is not new matter as it is supported by the Specification as found on page 6, line 15. Dependent claims 2-5 and 7 by virtue of their dependency contain all the limitations of their respective amended independent claims.

Additionally, the Examiner states:

> In claim 5, for the phrase "the protease is introduced" it is unclear whether the protease is being introduced into a reaction mixture in solution, into a cell, or into an organism. Clarification is required. For purposes of examination, it is assumed that the protease is being introduced into a cell.

Id. at. 4.

Claim 5 has been amended to recite "the protease is introduced into a cell" for clarification. Support is found in the application as filed.

The Examiner also states:

> In claim 6, the phrase "expressing [of] the recombinant fluorescent substrate in the presence of a plurality of proteases has three possible interpretations. Either the substrate is expressed in a single cell comprising a plurality of proteases, the substrate is expressed in a plurality of cells wherein each cell has a single substrate, or both of the above. For purposes of examination, it

> is assumed that the substrate can be expressed in a single cell comprising a plurality of proteases or the substrate can be expressed in a plurality of cells, wherein each cell has a single protease.

Id.

Claim 6 has been amended to more clearly indicate that the construct can comprise one or more (i.e., a plurality) protease substrate sequences. Support is found on page 8 of the specification.

Claim Rejections - 35 U.S.C. § 112 - First Paragraph

Claims 1-7 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the fluorescent protease substrate comprised of GFP1-157 fused to the N-terminus of a peptide having a substrate motif for the NS3/4A protease followed by GFP 158-238, does not reasonably provide enablement for a method using any fluorescent protease substrate comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter.

Applicants respectfully traverse this rejection. Other fluorescent proteins are presently known in the art such that one of skill in this art could create a construct having a fluorescent reporter other than GFP following the teachings of the specification. Moreover, having knowledge of the information taught in the specification, it would be reasonable for a skilled artisan to check known sources, in existence and known at the time of filing of the application, such that it would be no more than routine optimization to create a construct having a fluorescent protein that is a functional equivalent of the GFP.

Additionally, Applicants respectfully submit the specification is enabling for any protease substrate motif as the specification teaches that insertion of a protease-susceptible site into a surface exposed loop of an intrinsically fluorescent protein converts it into an intracellular

substrate for a protease and that proteolytic activity would be expected to generate two fragments. The presence of proteolytic activity leads to a protease-dependent quenching of the fluorescent protein. Therefore, the specification teaches a specific biochemical or biological process occurs in which the substrate is involved, i.e., proteolytic activity. Considering the level of skill and knowledge in the art, one skilled in the art would be able to practice the process steps of the invention. Applicants respectfully request that this rejection be withdrawn.

Claims 1-7 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner states:

> These claims are directed towards methods of assaying protease activity using a genus of fluorescent substrates comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. The specification teaches the structure of only a single representative species of such fluorescent substrates. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a fluorescent substrate comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter.

Id. at pp. 7-8.

Applicants respectfully traverse this rejection. The specification discloses that the protease substrate must be susceptible to protease activity and when cleaved has little intrinsic affinity to the fluorescent protein. Applicants respectfully request that this rejection be withdrawn.

Claim Rejections - 35 U.S.C. § 102

Claims 1-7 are rejected under 35 U.S.C. § 102(b) as being anticipated by Anderson et al.

Applicants respectfully traverse this rejection. Anderson fails to disclose the limitation of detecting a change in fluorescent quenching in the recombinant fluorescent substrate as an indication of protease activity (see claim 3). In contrast, Anderson discloses using GFP in fusion constructs to detect a presence of an altered phenotype of the cell itself (see column 23, lines 23-53). Moreover, in Anderson's preferred method, one must detect an altered phenotype, then the presence of the fusion protein is verified (see column 24, lines 60-62).

However, in Anderson's detection, as exemplified in Example 3, for instance, the relative fluorescent intensity of the fluorescence is calculated (i.e., averaged). Anderson does not detect a change in fluorescence. The relative fluorescence as measured by Anderson merely tells the reader the fluorescent intensity of the green fluorescent protein, not a change in intensity which in Applicant's method as claimed indicates whether cleavage between the reporter protein and the substrate has taken place. Therefore, Anderson fails to disclose all the claimed limitations. Applicants respectfully request that this rejection be withdrawn.

Claim Rejection - 35 U.S.C. § 103

Claims 1-7 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Mahajan et al. in view of Abedi et al.

Applicants respectfully traverse this rejection. There is no suggestion in the prior art represented by such references that they be combined in the manner proposed by the Examiner. Absent such a suggestion, there would be no reason why one skilled in the art who is faced with the same problem confronting the Applicants and who had no prior knowledge of Applicants' claimed method would consult the particular combination of references suggested by the Examiner. First, as stated by the Examiner, Mahajan does not teach a method for assaying protease activity using a fluorescent substrate, wherein the N-terminal portion of a fluorescent

reporter is fused to a peptide substrate followed by the C-terminal portion of the same reporter. More importantly, Mahajan fails to teach detecting protease activity by the quenching of the fluorescent wherein the fluorescent is quenched by cleavage in the protease substrate sequence. Instead, Mahajan teaches that upon cleavage of bifs and bYFs there is a decrease in the fluorescence resonance energy transfer (FRET) which is a technique used in analyzing intermolecular distances based on the transfer of energy from a donor molecule to an acceptor molecule without the omission of a photon. This is in contrast to Applicants' method where fluorescence is quenched as a result of proteolytic activity being present. This indicates cleavage in the fluorescent reporter protein into fragments. Moreover, Applicants teach that the quenching is measured by means such as fluorescent activated cell sorting (FACS), which is different from FRET. As stated above, with FRET, intermolecular distances based on the transfer of energy from a donor molecule to an acceptor molecule are analyzed. More importantly, FRET employs the transfer of resonance energy between two fluorophors. Therefore, two dyes will be present. Conversely, FACS, uses laser beams to detect differences in fluorescence between different types of cells in a mixture. Therefore, one of ordinary skill in the art would not likely use this combination of references alone or in combination with each other as suggested by the Examiner to arrive at the Applicants' invention.

Abedi fails to teach detecting the quenching in fluorescence as a result of proteolytic activity being present. In fact, Abedi teaches away from the Applicants' method which bases detection on proteolytic activity indicated by a decrease in fluorescence. On page 629, second column, second paragraph, Abedi states "in certain cases that may be helpful to select from the expression library those sequences that express the highest level of fusion protein as cells. Alternatively, it may be desirable simply to exclude all library constructs that do not express

scaffold levels above background." Abedi is in stark contrast to the present invention where quenching of fluorescence is used to detect activity of the cleaved substrate. Therefore, one of ordinary skill in the art would not likely use this combination of references alone or in combination with each other as suggested by the Examiner to arrive at the Applicants' invention. Therefore, this rejection must be withdrawn.

New Claims

Claims 14-29 are new. These claims are well-supported by the specification. Support for dependent claims 14, 20 and 25 can be found on page 6 of the specification. Support for independent claims 15, 21 and 26 can be found on page 4 of the specification. Support for claims 16-19, 22-24 and 27-29 can be found on page 7 of the specification.

Conclusion

This amendment adds two (2) independent claims over three (2 claims x $43.00). Also, this amendment adds three (3) dependent claims over twenty (3 claims x $9.00). Therefore, please charge Deposit Account No. 26-0084 an amount of $113.00.

No other fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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